TULARÆMIA Francis 1921.[1]

VII. SIX CASES OF TULARÆMIA OCCURRING IN LABORATORY WORKERS.

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All of the men, six in number, who have been intimately connected during the past two years with the laboratory investigations of tularæmia, which the Public Health Service has been conducting, have contracted this disease. Such a record of morbidity among investigators of a disease is probably unique in the history of experimental medicine. Fortunately, there were no fatalities. Two of the men contracted the disease in the field laboratory in Utah, where they were compelled to work under primitive conditions; the other four contracted the infection in the Hygienic Laboratory at Washington, D. C. Two of the men were physicians, with years of experience in working with infectious diseases and materials; one was a highly trained scientist; and the other three were experienced laboratory assistants.

Before discussing the diagnosis of tularæmia in these laboratory cases we will first summarize the picture presented by seven known cases of this disease which have occurred by natural infection in Utah. All seven had a sudden onset of illness with fever, closely following an insect bite, which became the site of suppuration and which was accompanied by a consequent unilateral suppurative lymphadenitis of the glands, which immediately drained the bitten area. The constitutional disturbance was severe, as indicated by febrile attacks which lasted from three to six weeks and which were followed by slow convalescence. *Bacterium tularense* was isolated from the suppurating lymph glands in five cases and from the blood in two. Serological tests were positive for complement fixation and agglutination, using antigens composed of cultures of *Bacterium tularense*. In an endemic focus no second attacks have come to our attention, although this subject was not especially investigated.

In reaching the diagnosis of tularæmia in the six infections contracted in the laboratory, the evidence will be considered in comparison with that of the seven infections contracted in nature in Utah, under the following heads: (1) Clinical evidence, (2) serological tests, (3) epidemiologic evidence, (4) absence of local lesions and the portal of entry of the infection, and (5) absence of *Bacterium tularense* from the blood.

1. CLINICAL EVIDENCE. (See Appendix A.)

The laboratory cases all had a sudden onset, with high fever, which, after remitting about the third day almost to normal, immediately became high again and then fell gradually to normal at the end of

[1] See Public Health Reports vol. 36, No. 30, July 29, 1921, pp. 1731-1753; vol. 37, No. 3, Jan. 20, 1922, pp. 83-115.

This series of seven articles on tularæmia will be combined and reprinted in pamphlet form as Hygienic Laboratory Bulletin No. 130.

about three weeks (see Charts 1 and 2). A lack of other significant constitutional disturbances or physical signs was noted. A slow convalescence extended over about two months, and recovery took place without complications.

2. SEROLOGICAL TESTS. (See Appendix B.)

Complement fixation and agglutination tests made on the serums of the six laboratory cases on several occasions, from January, 1921, to October, 1921, were all positive. The shortest interval after the onset of the disease before the serum was tested was 13 days; the longest interval from the date of illness was more than two years. Serums from two of our laboratory cases were found positive by comparison with serums from four known cases of tularæmia from which Francis had isolated the organism in Utah. These two serums served as positive controls in the tests made on the other laboratory cases. In all 66 negative control serums, for the most part from nonfebrile patients hospitalized in Washington, were used. Two or three of the latter gave some degree of positive complement fixation action but were negative by the agglutination test. We wish to point out that the control serums preferably should have been from patients in the febrile stages of well-known diseases, but such cases were not available. Seven of the 66 negative control serums were from laboratory personnel coming in casual contact with infected animals; these were completely negative. The control on Case 6 was unique and the most perfect one that could be obtained; his serum was tested on two occasions by complement fixation and agglutination during his exposure to the laboratory infection, but before the onset of his illness, and was negative by both tests, whereas after his illness it became strongly positive by both tests on two occasions.

3. EPIDEMIOLOGIC EVIDENCE.

The entire laboratory personnel (six) who have been employed continuously in handling or dissecting rodents infected with the Utah strains of *Bacterium tularense* have contracted febrile attacks which lasted approximately three weeks and were followed by slow convalescence. These attacks developed on the seventh, seventeenth, thirtieth, forty-third, eightieth, and ninety-eighth days, respectively, of such employment. Case 1 developed a second attack two years and five months after the first attack. Three of the cases have continued their work after recovery for many months in the same manner as before their illness without developing a second attack.

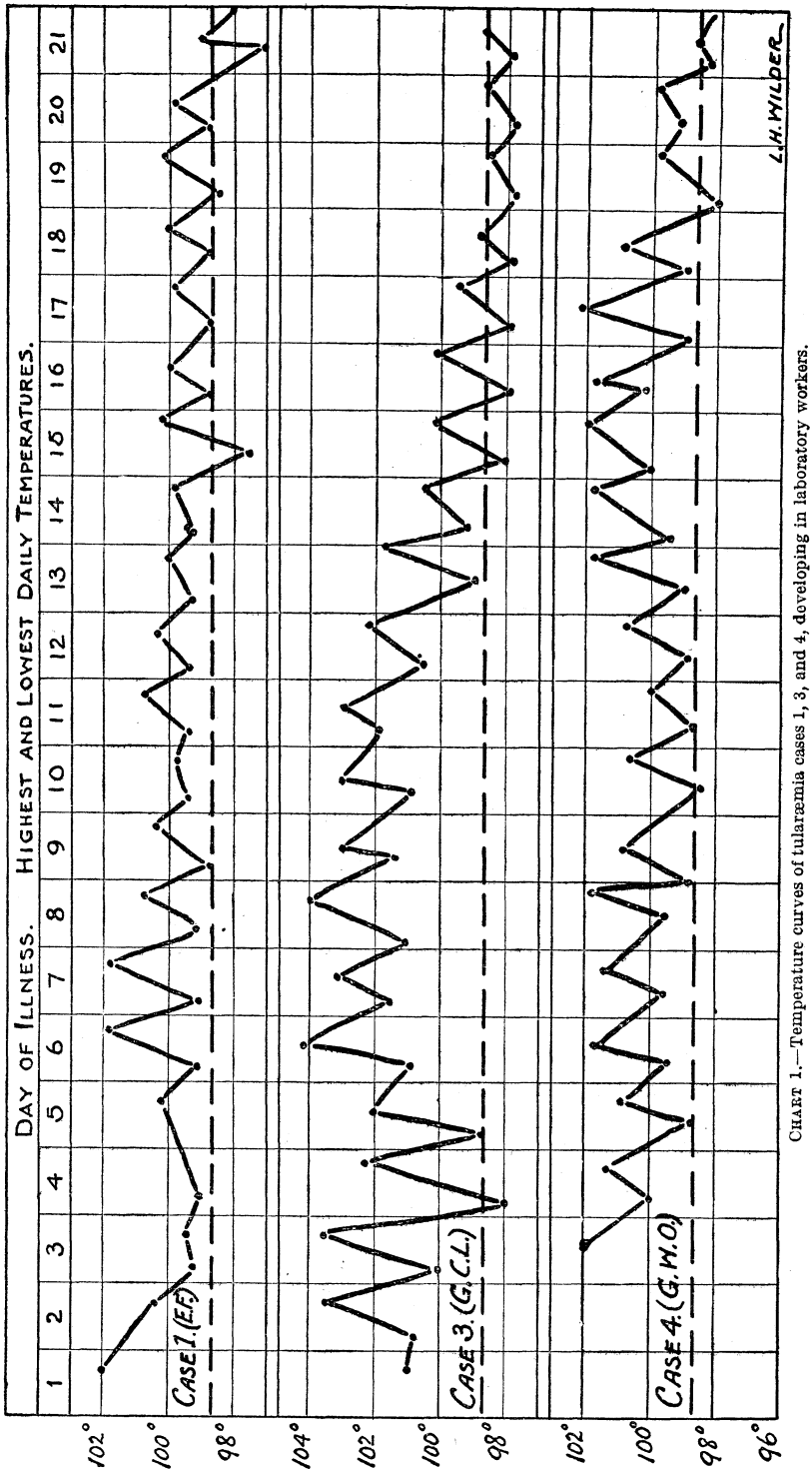

CHART 1.—Temperature curves of tularemia cases 1, 3, and 4, developing in laboratory workers.

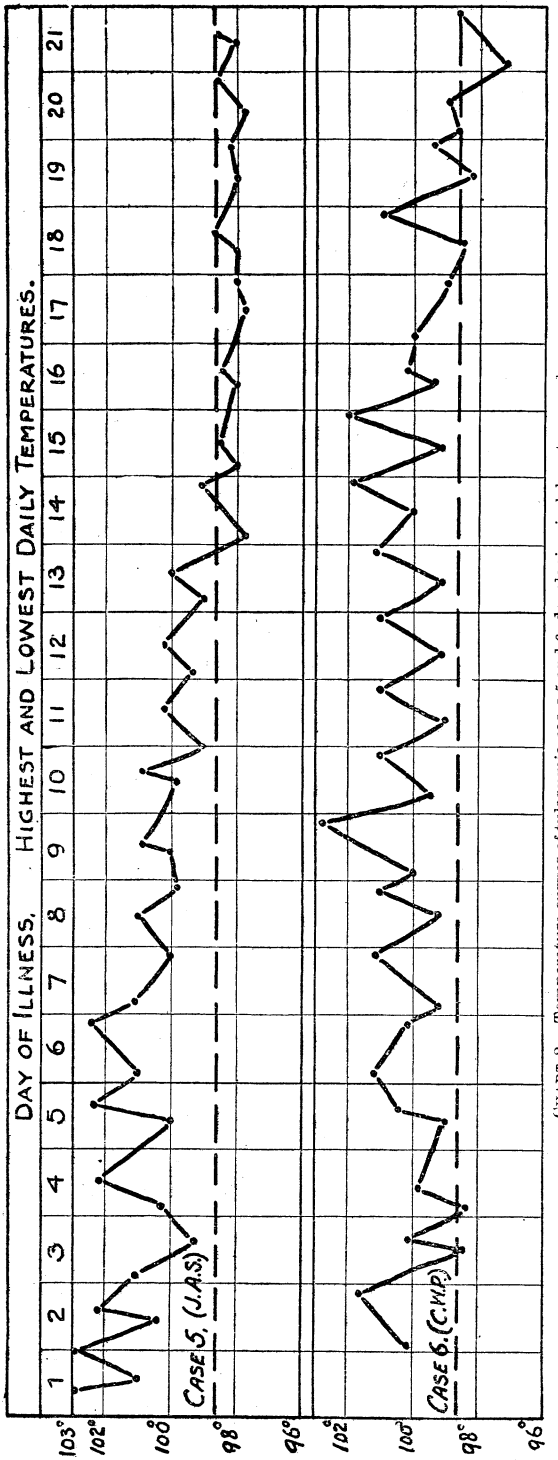

CHART 2.—Temperature curves of tularæmia cases 5 and 6, developing in laboratory workers.

The laboratory room in which animals were inoculated, dissected, and handled after autopsy was so located in the middle of the building that it was freely used as a passageway by other workers on the same floor and by general laboratory attendants. None of the laboratory personnel thus coming into casual contact with the work developed the disease, although several either worked with cultures or occasionally inoculated an animal. During the period when four cases developed in the laboratory in those who handled or dissected rodents, there was a remarkably low sick rate among the other personnel of the laboratory, numbering about 100, none of whom developed a febrile attack. Moreover, no infections occurred among fresh stock animals kept in the laboratory in cages adjacent to infected animals.

Infected insects whose bites have been followed by transmission of the infection to animals are: The bloodsucking fly, *Chrysops discalis;* the stable fly, *Stomoxys calcitrans;* the bedbug, *Cimex lectularius;* the squirrel flea, *Ceratophyllus acutus;* the rabbit louse, *Hæmodipsus ventricosus;* and the mouse louse, *Polyplax serratus.* Of the insects enumerated, only the first four are known in our experience to bite man.

Chrysops discalis can not be excluded as a factor in the transmission to Cases 1 and 2, who contracted the infection while working in the field laboratory in Utah, but can positively be excluded in the four cases which contracted the infection in the laboratory in Washington.

Stomoxys calcitrans might have been a factor in the Cases 1 and 2, developing in Utah, but three of the Washington cases developed during the season of minimal prevalence of this fly, during which time none was seen in the laboratory.

No fleas or bedbugs were seen in connection with the infected animals either in Utah or in Washington. None of the cases had any knowledge of being bitten by the insect carriers enumerated.

4. ABSENCE OF LOCAL LESIONS AND THE PORTAL OF ENTRY OF THE INFECTION.

The six laboratory cases (except second attack of Case 1) furnished no local lesions indicating the portal of entry of the infection and no involvement of superficial lymph glands. This is in contrast to the human cases of tularæmia which contracted the infection in nature in Utah, all of whom had a pronounced lesion at the site of infection (insect bite) and a consequent pronounced lymphadenitis of the adjacent glands; but it is in harmony with numerous observations on the disease in animals, both by natural infection and laboratory inoculation.

Francis has recently shown that the infection traversed the unclipped, unshaved, unabraded, and unrubbed skins of five guinea

pigs when spleen juice of infected guinea pigs was gently placed on the skin of these animals after turning aside the hair on their backs. The experimental guinea pigs all wore a stiff collar 1½ inches wide, which served to sufficiently immobilize the head to prevent ingestion of the infected material. The animals all died acutely. The local lesion consisted of a pale papule surrounded by slight congestion. The secondary lymph glands were caseous, and the spleen and liver showed the typical lesions of the disease.

White mice injected with blood subcutaneously or bitten by infected bedbugs may die from the infection and yet show almost no appreciable lesion at the site of infection or in the superficial lymph glands.

Bacterium tularense was isolated at autopsy by guinea pig inoculations from the spleens of 17 jack rabbits infected in nature in Utah, in which an absence of involvement of the inguinal and axillary glands was noted.

In transmission experiments conducted in Utah upon rabbits and guinea pigs, instances were noted of the absence of a lesion at the site of an infected *Chrysops discalis* bite or in the adjacent lymph glands, whereas the liver or spleen showed typical lesions.

We have noted instances of the absence of involvement of the subcutaneous glands of guinea pigs after subcutaneous injection with infected bedbug feces which had dried 20 days on a filter paper; yet the guinea pigs died acutely with lesions of the spleen and liver typical of tularæmia.

In view of the facts stated in preceding paragraphs, consideration must be given to the skin of the hands as a possible portal of entry of the infection in laboratory workers, even in the absence of a local lesion or lymphadenitis.

On the other hand, in the second attack which Case 1 of our series developed two years and five months after his first attack, there was a papule on the finger from which *Bacterium tularense* was isolated by guinea pig inoculation. There was also a secondary lymphadenitis involving the epitrochlear and axillary glands of the same arm, but an absence of constitutional symptoms.

5. ABSENCE OF BACTERIUM TULARENSE FROM THE BLOOD.

The blood of three of the laboratory cases, taken during the febrile stage, was injected intraperitoneally into guinea pigs, but with negative results. The absence of the organism from the blood in these cases as shown by guinea pig inoculations is taken as an indication of the mildness of the attacks. Of the two cases in Utah in which the organism was isolated from the blood, one terminated fatally and the other was very sick. The presence of the organism in the blood

probably indicates a grave condition in which the patient's resistance has given way. Laboratory animals which uniformly die from the infection show the organism with great constancy in the blood in the later stages.

UNRECOGNIZED CASES OF TULARÆMIA.

Known foci of this infection in rodents have been reported from California, Utah, and Indiana. The known insects capable of transmitting the infection in animals are two species of biting flies, one species of fleas, two species of lice, and the common bedbug. There are probably other foci and other transmitting insects in the United States. The most practical method of search for unrecognized cases of this disease is the routine testing of specimens of blood collected from various parts of the country for complement fixation and agglutination using an antigen consisting of *Bacterium tularense*. Our laboratory cases show well-marked antibodies to this antigen for many months after recovery.

As an instance of unrecognized cases of tularæmia, we wish to refer to the work of McCoy and Chapin,[2] of the Public Health Service, who discovered *Bacterium tularense* in 1912 as the cause of a plaguelike disease of rodents in the California ground squirrels.

They reported at that time complement fixation and agglutination to *Bacterium tularense* antigens not only in the case of serums of naturally or artificially immune animals but also in the case of 2 out of 11 human serums tested. The two positive human serums were from Dr. C. W. Chapin and a laboratory attendant, both of whom were extensively engaged in handling or dissecting infected rodents in the laboratory.

Dr. Chapin now states that shortly previous to testing his own serum in 1912 he had had a febrile attack which kept him off duty for about three weeks and which was unaccompanied by glandular enlargement or other local lesion. No absence from duty on the part of the laboratory attendant can now be recalled by Dr. Chapin.

In the light of present knowledge it seems certain that what to McCoy and Chapin was a puzzling circumstance (the presence of antitularense amboceptors in the serums of two laboratory workers) was the proof of two unrecognized human cases of tularæmia.

SUMMARY AND CONCLUSIONS.

All of the persons (six) who have been intimately engaged during the past two years in the laboratory in handling or dissecting rodents infected with the Utah strains of *Bacterium tularense* have suffered an attack of tularæmia.

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[2] McCoy, G. W., and Chapin, C. W., *Bacterium tularense*, the cause of a plaguelike disease of rodents. Public Health Bulletin, No. 53, 1912, p. 21.

The diagnosis in each of the six cases rests upon the occurrence of a febrile period lasting about three weeks, positive serum reactions for agglutination and complement fixation to antigens composed of *Bacterium tularense*, and the absence of febrile attacks in 100 other persons in the laboratory coming in casual contact with the infected rodents.

Consideration must be given to the skin of the hands as a possible portal of entry of the infection in laboratory workers even in the absence of a local lesion or lymphadenitis.

A second attack has recently occurred in Case 1 of the above series, two years and five months after his first attack. The second attack was associated with evident cracks on the fingers, on one of which there developed an inflammatory papule which was soon followed by enlarged, painful, and tender lymph glands in the epitrochlear and axillary regions of the corresponding side, but without fever or other constitutional disturbance. *Bacterium tularense* was isolated from the papule by guinea pig inoculation.

The absence of constitutional symptoms in the second attack, although there was a local lesion and consequent lymphadenitis, is accounted for by the persistence of immune bodies acquired by the first attack.

Unrecognized cases of tularæmia probably occur in the known foci of infection in the United States, some of which may have febrile attacks without local lesions, while some may have local lesions and a secondary regional lymphadenitis without very notable constitutional disturbance.

Routine serological tests for agglutination especially and for complement fixation using antigens composed of *Bacterium tularense* would probably not only detect cases in known foci of infection but would bring to light unknown foci. Positive serological reactions are known to persist for two years after an attack. Light might be thrown upon the etiology of some fevers of undetermined origin.

A warning is sounded against unwarranted indifference to an infection which, in our experience, has claimed all of those who have persistently worked with it in the laboratory.

Acknowledgment.—Through the courtesy of the United States Naval Hospital, Washington, D. C., Case 3 was treated in that institution on the service of Lieut. Commander J. J. O'Malley, Medical Corps, United States Navy, to whom we are indebted for clinical data on this case.

To Dr. J. J. Bateman, Passed Assistant Surgeon (R.), United States Public Health Service, we are indebted for clinical data on Cases 4, 5, and 6, who were treated in United States Public Health Service Hospital No. 32, Washington, D. C.

Appendix A.

BRIEF CLINICAL REPORTS OF SIX LABORATORY CASES OF TULARÆMIA.

CASE 1.

First attack.—E. F., male, age 49, physician, began investigations of tularæmia in Delta, Utah, July 23, 1919. His exposure differs from the other cases to be reported in that in addition to exposure to laboratory animals he took blood and pus on two occasions from a human case which terminated fatally. On the thirtieth day of his investigation, August 23, 1919, E. F. became ill in the late afternoon, feeling tired and weak and having a temperature of 102.2°. With the exception that his temperature (see temperature curve, Chart 1, Case 1) almost reached normal on the third and fourth days, at which time he felt slightly improved; his fever continued until the twenty-fourth day. During the first 12 days of his illness he packed up his laboratory equipment and animals in Utah with great difficulty and proceeded with them to Washington, D. C., and after his arrival made a futile attempt to continue work. The next 14 days he spent in the hospital lying on the bed, but not confined to the bed.

The temperature was highest, 102.2°, on the first day and showed a steady decline to normal on the twenty-fourth day. The departure of the patient from the hospital on the twenty-eighth day was attended with some forced exercise, which resulted in a secondary rise of temperature which lasted four days, after which it remained normal.

The second month was spent in a hotel, lying on the bed most of the time. The third month was one of slow convalescence.

Throughout the illness there was an absence of localized pain or tenderness, except that on the sixteenth day of illness a sore throat developed on the right side, manifested by redness of the anterior pillar of the fauces without involvement of the tonsil. Practically the only complaint was that of languor, or weakness, and a desire to remain quiet on the bed.

Blood: White cell count on the fifteenth day, 13,600; white cell count on the twenty-first day, 8,650.

Agglutination tests for typhoid, paratyphoid A and B, on the twenty-first day were negative.

Serological tests for tularæmia, made January 20, April 29, June 15, and September 30, 1921, were all positive. (See Tables I, II, IV, and V.)

Second attack.—Following recovery from the first attack, this patient continued handling and dissecting infected guinea pigs, rabbits, and white mice in the laboratory for two years without using gloves. During this time infected material frequently got on his hands, but was washed off. In the first part of January, 1922, he

handled formaldehyde excessively in preparing specimens for preservation in Kaiserling solutions, and very evident cracks appeared on the fingers of both hands. In spite of this he autopsied infected animals without gloves.

On January 14 the right index finger showed on the inside of the first phalanx, near its upper end, a red, tender papule at the site of a recent crack. That night attention was directed to enlarged, tender lymph glands located in the right epitrochlear and right axillary regions.

From January 15 to 20 the glands mentioned were painful, tender, and noticeably enlarged on inspection and palpation. There was a red flush of the skin overlying the glands and at the outer border of the right biceps muscle, but no red streaks were noted on the hand, forearm, or arm.

On January 21 no redness could be noted, and the glands were not painful, but were still tender and enlarged on palpation.

The temperature was taken daily throughout the attack, but was never above normal. No notable constitutional disturbance was observed and the patient continued work as usual. On the seventh day the white blood cells numbered 7,500, and 30 c. c. of blood taken from the median basilic vein were injected intraperitoneally into six guinea pigs with negative results.

On the second day of the attack the papule on the finger was incised, but no pus was noted. The escaping blood was injected subcutaneously on the right side of the abdomen of a guinea pig. The papule was swabbed with iodine and dressed with wet bichloride of mercury dressings for the next five days, during which time no pus was noted in the wound.

The guinea pig was dying on the fifth day after injection and was chloroformed. It showed a severe local reaction at the site of injection and typical gray granular caseation of the right inguinal, right retroscapular, and retropancreatic lymph glands. Its liver and spleen were studded over the surface with small foci or granules of necrosis. Portions of the lymph glands and spleen of the guinea pig were rubbed on the shaved, abraded skin of the abdomen of two healthy guinea pigs, causing acute death with the typical lesions of tularæmia.

CASE 2.

B. M., male, age 37, scientific expert, stated that on July 20, 1920, seven days after beginning work in the field laboratory at Delta, Utah, which work brought him into intimate contact with laboratory animals suffering with, or dead from, tularæmia, he became ill rather suddenly. His chief symptoms at the time were headache, backache, shifting pains involving especially the chest, knees, and elbows, and

fever. His temperature was not taken at the time, but from July 22 to July 31 it ranged from 100° in the morning to 103° in the evening, after which it reached normal in the morning and was no longer taken. Malaria was suspected, but examinations of the blood for parasites were negative. He remained at work most of the time, although barely able to get about. The shifting pains persisted for about a month, during which time there was some loss of appetite and gastro-intestinal disturbance, with a weight loss of 15 pounds. On August 19 he took 10 days' sick leave, during which he spent most of the time lying on the bed. After this he returned to duty, but stated that it was three or four months before he was able to perform his work without undue fatigue, and that for more than a year afterwards he has been troubled with pains in the back.

On June 14, 1921, the patient happened to be at the Hygienic Laboratory, and a sample of his serum was obtained, which was tested for tularæmia antibodies, by both the complement fixation and agglutination reactions. In both tests the results were positive. (For protocols of tests see Tables IV and V. Temperature curves are not given, as complete temperature records were not kept.)

CASE 3.[3]

G. C. L., physician, age 37, engaged in experimental investigations of tularæmia at the Hygienic Laboratory, Washington, D. C., was in good health up to October 23, 1920 (43 days after beginning this work), when, after putting in a full day at the laboratory, he suddenly became ill in the evening. He was compelled to go to bed because of weakness and dizziness, and a few minutes later had a fairly severe chill, after which the temperature was found to be 101°. (See temperature curve, Case 3.) The temperature, which was quite irregular, gradually became higher, reaching 104.2° on the sixth day. There was a remission to 98° the morning of the fourth day, at which time the patient got up with the intention of going to work, but suddenly became dizzy and weak and had to go back to bed. On the eighth day he was taken to the hospital, where the temperature, after reaching 103° for the next three days, gradually began to fall, reaching normal on the seventeenth day, and, with the exception of a slight rise a few days later, remained normal. The pulse was fairly rapid, ranging from 80 to 98, and remained high for some time after the fever dropped. The blood pressure, taken on several occasions, was normal. During the first two weeks there was a moderately severe rhinitis, the secretions being at times blood tinged, and on two occasions a slight epistaxis occurred. There were no pains at any time, only a desire to be quiet and sleep a great deal, and occasionally there was slight nausea. Repeated physical examinations were

[3] This case is also reported by Lieut. Commander J. J. O'Malley, Medical Corps, U. S. Navy, in the Journal of the American Medical Association, 1922, vol. 78.

practically negative. The treatment was absolute rest in bed and careful feeding and nursing. He was discharged from the hospital November 29, having lost only 15 pounds in weight.

After returning home the patient spent a month resting most of the time. Temperature of about 100° was noted several times during the first 10 days at home. By the end of the month he could walk a half mile without much fatigue. The only special symptoms were the development at different times of localized hyperesthetic areas of the skin (the sensation being that of a mild burn, but with no visible lesion), and an attack of mild tympanitis lasting more or less continuously, except while patient slept, for about 48 hours.

He returned to work January 1, but for the first month usually went home at noon and spent most of the afternoon in bed. It was late in the spring before he had regained a condition approximating normal health. Transient pains in the calves of the legs, gradually becoming milder and occurring less frequently, have persisted for more than a year.

Laboratory examinations made on Case 3.

October 26: White cell count, 12,000; nasal secretions blood tinged injected into guinea pigs with negative results.

October 28: Blood culture for typhoid negative.

October 30: Blood culture for typhoid negative. Widal positive for typhoid, negative for paratyphoid A and B. (Patient had received three injections of single typhoid vaccine late in 1914.) Inoculation of a guinea pig with 5 c. c. blood intraperitoneally resulted negatively.

October 31: White cell count, 8,300, red cells, 5,900,000, differential not significant.

October 30 to November 20: Several examinations of urine and feces for *B. typhosus* were made with negative result.

January 20, April 29, May 11, June 15, and September 30, 1921: Serological tests for tularæmia were all positive. (See Tables I to VI.)

CASE 4.

G. W. O., male, age 36, laboratory assistant in connection with investigations with experimental tularæmia. On April 9, 1921, after having been engaged in this work 98 days, he was taken suddenly ill. He had not felt well in the forenoon and at 3 p. m., while at work, was suddenly seized with a sharp pain over the right shoulder, radiating downward with the spine and localizing near the twelfth dorsal vertebra. On reaching home, only a short distance away, the pain radiated to the lumbar region and later to the muscles and joints of the legs. The pains continued to shift, at times involving the eyeballs, superciliary ridges, and occipital regions. He remained at home for a week, continuing to have shifting pains and temperature, which, after dropping to normal during the forenoon of the fourth day, gradually became higher, accompanied by a feeling of increasing

weakness. During this time physical examination was practically negative. On the seventh day he was taken to the hospital, where a carefully conducted and complete physical examination was negative, except that the areas in which he complained of pain were sensitive or tender, namely, occipital region, muscles of neck, superciliary ridges, and vertebral border in the lumbar region.

Treatment was absolute rest in bed and symptomatic. Temperature (see Chart 1, Case 4) reached normal on the twenty-first day. Patient continued to have pains in the head, muscles, and joints until the sixteenth day. During the febrile stage his pulse range was from 70 to 80, reaching 90 April 22. After the febrile stage, the average was about 75. (This patient normally has a slow pulse, which now averages about 66, when sitting.)

Laboratory examinations Case 4.

Feces: Examined for *B. typhosus* with negative result on May 4, 11, and 14.

Blood: White cell count 6,400 on April 15; 8,770 on April 28, when red cells were 5,000,000, differential about normal.

Blood cultures: Made April 15 and April 23, designed to show the presence of *Bacterium tularense*, *B. typhosus*, streptococcus, etc., on the following mediums: Glucose blood agar slants and plates, 1 per cent glucose agar, special egg medium of McCoy and Chapin, Levinthal's cooked blood agar, and in graded amounts into a series of tall test tubes, each containing 50 c. c. of bouillon (great care being taken not to jar the tubes and disturb the filamemts of fibrin). No growth was obtained on any of the mediums.

Inoculations of 7 guinea pigs April 15, and 5 guinea pigs April 23, each receiving intraperitoneally 4 c. c. of blood plus 4 c. c. of saline, all resulted negatively for tularæmia.

Immunological tests: Widal was slightly positive for *B. typhosus*, negative for paratyphosus *A* and *B*. (Patient had received three injections of single typhoid vaccine in November, 1913.) Agglutination and complement fixation tests for tularæmia, April 29, May 11, June 15, August 5, and September 30, 1921, were all positive. (See Tables II to VI.)

CASE 5.

J. A. S., male, age 29, succeeded G. W. O. (Case 4) as laboratory assistant with the tularæmia investigation. On April 28, 1921, the 17th day of his exposure to infected animals, after working till 11 a. m., he complained of not feeling well and of being chilly. Temperature, taken at once, was 103°, pulse 100, respirations 24; otherwise physical examination was negative. During the next half hour, while waiting for the ambulance to take him to the hospital, he had a fairly severe chill. History taken on his admission to hospital shows that he complained of headache, shifting pains in the muscles and joints, weakness, and anorexia. Physical examination was negative, except that the areas in which he complained of pain were found to be either hypersensitive or tender, and that there was a slight impair-

ment of resonance over the right scapular region. Blood pressure was normal.

Patient's temperature (see Chart 2, Case 5), after dropping almost to normal on the third day, continued high until the sixth day, after which there was a gradual drop to normal on the thirteenth day. This was the only one of our cases who was taken immediately to the hospital on the onset of symptoms, which may account for the shorter febrile stage. His pulse range during the febrile stage was from 80 to 100, and during the next two weeks about 80, after which it dropped to 70.

He complained of headache and muscular pains a great deal during the first few days, and, to some extent, for the first two weeks. He was discharged May 29, 18 days after his temperature became normal, and remained at home gradually improving until July 4, when he was almost instantly killed in a railway accident. A complete post-mortem examination failed to show any evidence of lesions of tularæmia either active or healed. All the organs and tissues were normal except for the crushing injuries produced by the accident.

Laboratory examinations made on Case 5.

Feces: Negative for *B. typhosus*, May 5, 11, and 14.

Blood: Cultures made on April 28, as was done in Case 4, except that in addition fermentation tubes were used, all negative.

Inoculations intraperitoneally of 7 guinea pigs on April 28, and 5 more guinea pigs May 10, each receiving 4 c. c. of defibrinated blood plus 4 c. c. of saline, gave negative results for tularæmia.

Immunological tests: Agglutination and complement fixation tests for tularæmia, made on May 10 and June 15, 1921, were positive. (See Tables III, IV, and V.)

CASE 6.

C. W. P., male, age 29, succeeded J. A. S. (Case 5) as laboratory assistant in tularæmia investigations. On July 17, 1921, 80 days after beginning this work, he felt a severe pain in his left elbow just after going to bed. This pain lasted only a few minutes and was followed by a chill lasting about 10 minutes. The following day he felt weak, had no appetite, had a headache of moderate severity, and was in this condition when first examined at his home, July 19. A partial physical examination conducted at the time revealed nothing of importance except temperature 101.8°, pulse 100, respiration normal. He was taken to the hospital the same afternoon, where a complete physical examination was also practically negative.

Examination of temperature curve (Chart 2, Case 6) shows that on the mornings of the third and fourth days patient's temperature reached normal. At this time he said that he did not feel sick enough to stay in bed. After that his temperature began to rise and remained fairly high (highest 102.7° on July 26) until the six-

teenth day, after which it began to fall, becoming normal on the twentieth day. His pulse during the febrile stage was variable, ranging from 80 to 100; after the febrile stage it averaged about 80. He complained of nothing at any time except weakness and occasionally some nausea. He was discharged August 21, 14 days after his temperature became normal. He remained at home slowly convalescing until October 1. For the next month he worked in the laboratory during the forenoons and rested most of the afternoons. Since that time he has been on duty full time. His only complaint since going to work has been that of a dull pain in the left side, which at first bothered him a great deal, but which has now almost entirely disappeared.

Laboratory examinations made on Case 6.

Blood: White cells 7,300, red cells 5,000,000, differential unimportant July 19.

Inoculations of 7 guinea pigs on July 22 and of 5 pigs August 5, each with 4 c. c. of defibrinated blood plus 4 c. c. of saline intraperitoneally, gave negative results for tularæmia.

Immunological tests for tularæmia made August 5 were positive. (See Table VI.) Further tests made September 30 by both agglutination and fixation methods were also positive. (Protocols not given.)

Appendix B.

SEROLOGICAL REPORTS.

DISCUSSION OF TABLE I.

On January 20, 1921, complement fixation tests were made (1) to determine whether serums collected after recovery from naturally infected human cases of tularæmia would give a definite reaction with *Bacterium tularense* antigen; (2) to determine whether serums from human cases 1 and 3 originating in the laboratory would react positively; and (3) to determine whether serums from control persons, presumably uninfected, would fail to react.

The serums from the naturally infected cases definitely known to be tularæmia were collected by Francis September 28, 1920, and were from cases from which he had isolated *Bacterium tularense* (see Public Health Reports, vol. 36, No. 30, July 29, 1921, pp. 1731–1738). These serums were heated 30 minutes at 56° C. at time of collection and preserved by adding an equal amount of glycerin. Serum from laboratory Case 1 was obtained January 19, 1921, about 17 months after the onset of illness; serum from laboratory Case 3 was obtained January 19, 1921, about three months after the onset of illness. The control serums used in this test were from samples sent in for routine Wassermann tests.

The antigens used were saline suspensions of *Bacterium tularense* made by washing off the 72-hour growth on egg medium slants with small amounts of saline, care being taken to avoid breaking the

surfaces, and then heating the suspensions for 30 minutes at 54° C. No preservative was added. Three separate antigens were prepared; one was from a strain isolated from a ground squirrel by Passed Asst. Surg. W. T. Harrison in California in May, 1920, and the other two were isolated by Francis in Utah, one from a jack rabbit and one from a typical human case (G), whose serum was also used in the tests.

Tests had previously been made of other antigens prepared as above to determine whether fixation occurred with pooled Wassermann positive and pooled Wassermann negative serums, with negative results. The antigens used in this test had been titrated to determine suitable units for use in these experiments.

The results obtained are shown in Table I. It will be noted that very definite positive reactions were obtained with the four known positive serums and also with the serums from laboratory Cases 1 and 3 against all three antigens used. It is unfortunate that higher dilutions were not added, particularly in the series with the California strain antigen, so that the positive serums would be carried out to extinction of fixation; but even the old Utah serums, which were anticomplementary (see the no-antigen controls) in 1: 20 dilutions, fixed complement in 1: 540 dilution with at least one of the antigens. The nine control serums used gave negative results, with the exception of 7561, which can well be explained by the degree of anticomplementary effect present, and 7530, which gave a fairly strong fixation with one antigen and practically no fixation with the other two antigens. No more of serum 7530 was available for further tests. The positive serums all reacted definitely with suspensions of *Bacterium tularense* of squirrel, rabbit, and human origin, suggesting that the organism from these three sources is the same.

DISCUSSION OF TABLE II.

On April 29, 1921, agglutination tests were made to determine (1) whether serums from laboratory Cases 1 and 3, positive by the complement fixation test in Table I, would be positive by the agglutination test, and (2) whether serum from laboratory Case 4, taken on the thirteenth day of his illness, contained agglutinins. Six serums from hospital patients suffering with mild disorders unrelated to tularæmia were used as controls.

The antigen used was prepared from human strain G, in the same manner as described in the discussion of Table I, except that the suspension was heated 30 minutes at 56° C. and then preserved by the addition of 0.3 per cent tricresol. This antigen, designated G–32, was sealed in glass ampules and used in all the subsequent tests for agglutination and complement fixation.

The results (Table II) show that serum from laboratory Case 4, taken on the thirteenth day of illness, was positive. Serums, both unheated and heated, from laboratory Cases 1 and 3, which had been kept in the ice box over three months, but in neither case with preservative, gave about the same degree of positive reaction as fresh serum from laboratory Case 3. The controls failed to give any agglutination.

DISCUSSION OF TABLE III.

On May 11, 1921, tests were made by both the complement fixation and agglutination reactions (1) to compare the results of these two methods and (2) to determine whether serum from laboratory Case 5 (onset of illness Apr. 27, 1921) was positive. Serums from laboratory Cases 3 and 4, already found positive by previous tests, served as positive controls, and serums from three other men in the laboratory served as negative controls. Control serum A was from the man who later was the sixth laboratory case of our series. He had been working with animals infected with *Bacterium tularense* since April 28, 1921, but did not contract the disease until July 17, 1921. The results show that serum from laboratory Case 5 was positive on the fourteenth day of his illness. Both tests gave satisfactory results. The control serums were negative throughout.

DISCUSSION OF TABLE IV.

On June 15, 1921, serums from the five laboratory cases which had occurred up to that time were tested by the complement fixation method in comparison with (1) serums of several of the laboratory personnel, including serum A, from C. W. P , who, 32 days after this test, contracted tularæmia and became laboratory Case 6 of our series; (2) a serum from an immunized rabbit with high antityphoid titre, and a serum from a known case of typhoid with a positive Widal; and (3) 27 serums from ordinary hospital cases from two Government hospitals. The serums were collected on the day preceding the test and were not heated. The serums of the five laboratory cases were positive; Case 1 in dilutions up to 1 in 200; Cases 2, 3, and 5 in dilutions up to 1 in 400; Case 4 in dilutions up to 1 in 1,000.

The serum of Case 1 was taken 22 months after the attack of tularæmia. Of the 35 control serums, 27 were completely negative. Four, Nos. 6, 25, 31, and 34, can be classed as probably negative on account of being anticomplementary or reacting only in dilutions too low to be regarded as significant. The remaining four serums, Nos. 10, 13, 18, and 27, may be regarded as more or less positive, as the first three of them reacted in dilutions as high as our weakest positive control. These three, Nos. 10, 13, and 18, were therefore further

tested by the agglutination method (see Table V, with discussion). There was none of No. 29 remaining or it also would have been tested.

There is a possibility that some of the questionable positives with the complement fixation test would have been avoided had the serums been heated.

DISCUSSION OF TABLE V.

On June 16, 1921, serums Nos. 10, 13, and 18, which were found more or less positive by the complement fixation test on the preceding day and have been referred to in the discussion of Table IV, were submitted to the agglutination test. All serums tested were remaining portions of serums tested on the previous day; serums from laboratory Cases 1–5 served as positive controls; serums from controls 1–5 served as negative controls. The positive controls all reacted positively; the negative controls all reacted negatively, with the exception that control serum 1 gave some agglutination in the third and fourth dilutions, but not in the first two dilutions; the serums under investigation, Nos. 10, 13, and 18, all reacted negatively.

This result tends to confirm some previous observations (not recorded here) which we have made that the agglutination test is more reliable in that it is more specific than the complement fixation test for the detection of *Bacterium tularense* antibodies.

DISCUSSION OF TABLE VI.

On August 5, 1921, agglutination tests (see Table VI) were carried out on serum of laboratory Case 6, 19 days after the onset of his illness. This serum, as well as those from positive controls (Cases 3 and 4) gave definitely positive results. Control serum A (see Tables III, IV, and V) was from the laboratory attendant who became laboratory Case 6 of our series. The tests show that his serum reacted negatively on the thirteenth and forty-eighth days of his exposure to infected laboratory animals; but, having contracted the infection on the eightieth day, his serum reacted positively to the agglutination test 19 days after the onset of illness. His serum, shown to be definitely positive by this test, was subsequently tested October 1 by both the complement fixation and agglutination tests (protocols not given) in comparison with three positive and nine negative control serums, all taken on the same date and heated 30 minutes at 55° C. His serum was at this time somewhat more strongly positive than that of the positive controls. The negative controls remained negative throughout.

The serological tests in Case 6 are particularly significant in that they were negative before his illness and positive afterwards, the same antigen being used in all the tests.

TABLE I.—*Complement fixation tests of serums from four known positive cases of tularæmia, two of the laboratory cases here reported, and nine negative human controls. Saline suspensions of Bacterium tularense of squirrel, rabbit, and human origin were used as antigens. Tests were made Jan. 20, 1921.*

Serum.	Serum dilutions.																					
	California squirrel strain antigen.						Utah rabbit strain antigen.						Human strain G. antigen.						No-antigen controls.					
	No serum.	1:10	1:20	1:60	1:180	1:540	No serum.	1:10	1:20	1:60	1:180	1:540	No serum.	1:10	1:20	1:60	1:180	1:540	No serum.	1:10	1:20	1:60	1:180	1:540
Utah human cases:[1]																								
C	−	4+	4+	4+	+	−	−	4+	4+	4+	4+	−	−	4+	4+	4+	4+	4+	−	4+	3+	−	−	−
G	−	4+	4+	4+	4+	3+	−	4+	4+	4+	4+	4+	−	4+	4+	4+	4+	−	−	4+	4+	−	−	−
McK	−	4+	4+	4+	3+	2+	−	4+	4+	4+	3+	−	−	4+	4+	4+	2+	−	−	4+	+	−	−	−
S	−	4+	4+	4+	4+	2+	−	4+	4+	4+	4+	2+	−	4+	4+	4+	4+	+	−	4+	+	−	−	−
Laboratory human cases:[2]																								
Case 1	−	3+	3+	4+	4+	3+	−	3+	2+	3+	3+	+	−	3+	3+	3+	3+	−	−	−	−	−	−	−
Case 3	−	4+	4+	4+	4+	4+	−	4+	4+	4+	4+	4+	−	4+	4+	4+	4+	3+	−	−	−	−	−	−
Negative controls:[3]																								
7610	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
7570	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
7609	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
7560	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
7561	−	3+	+	−	−	−	−	3+	3+	3+	−	−	−	3+	+	−	−	−	−	2+	+	−	−	−
7622	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
7566	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
7564	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
7530	−	−	−	−	−	−	−	3+	3+	3+	+	−	−	+	+	−	−	−	−	−	−	−	−	−

[1] These serums were obtained in Utah on Sept. 28, 1920, from four recently recovered cases of tularæmia which had received their infection in nature and from which *Bacterium tularense* had been isolated during their illness. The serums were heated at time of collection for 30 minutes at 56° C. and subsequently preserved by adding an equal amount of glycerin. In the lower dilutions of the no-antigen controls, these four serums were anticomplementary.

[2] Serums from laboratory cases 1 and 3 were taken on the day preceding the test, about 17 months and 3 months, respectively, after the onset of illness.

[3] These control serums were from samples sent in for Wassermann tests.

TABLE II.—*Agglutination tests of serums from laboratory cases 1, 3, and 4, and six human controls, using Bacterium tularense antigen G 32. Test made Apr. 29, 1921.*

Serum.	Date serums were obtained.	Serum dilutions.							Remarks.
		No serum.	1:10	1:20	1:50	1:100	1:500	1:1000	
Laboratory cases:									
Case 1	Jan. 19	−	+	+	+	+	−	−	Serum not heated.
Case 3	...do....	−	+	+	+	+	−	−	Do.
Case 1	Jan. 24	−	+	+	+	−	−	−	Heated 56° ½ hour.
Case 3	...do....	−	+	+	+	+	−	−	Do.
Case 3	Apr. 27	−	+	+	+	+	−	−	Serum not heated.
Case 4	Apr. 22	−	+	+	+	−	−	−	Do.
Control serums:									
No. 1	Apr. 26	−	−	−	−	−	−	−	Heated 56° ½ hour.
No. 2	...do....	−	−	−	−	−	−	−	Do.
No. 3	...do....	−	−	−	−	−	−	−	Do..
No. 4	...do....	−	−	−	−	−	−	−	Do.
No. 5	...do....	−	−	−	−	−	−	−	Do.
No. 6	...do....	−	−	−	−	−	−	−	Do.

TABLE III.—*Comparison of complement fixation and agglutination tests made on laboratory cases 3, 4, and 5. Test made May 11, 1921. Antigen used, G 32.*

Serum.	Date collected.	Complement fixation.								
		Serum dilutions.								
		[1] 1:10	1:20	1:50	1:100	1:200	1:500	1:1000	1:2000	1:4000
Laboratory cases:										
Case 3	May 10	−	4+	4+	4+	4+	4+	−	−	−
Case 4	...do....	−	4+	4+	4+	4+	2+	−	−	−
Case 5	...do....	−	4+	4+	4+	4+	−	−	−	−
Control serums: [2]										
A	...do....	−	−	−	−	−	−	−	−	−
No. 1	...do....	−	−	−	−	−	−	−	−	−
No. 2	...do....	−	−	−	−	−	−	−	−	−

Serum.	Date collected.	Agglutination.							
		Serum dilutions.							
		1:10	1:20	1:50	1:100	1:200	1:500	1:1000	1:2000
Laboratory cases:									
Case 3	May 10	2+	2+	2+	2+	−	−	−	−
Case 4	...do....	2+	2+	2+	2+	+	−	−	−
Case 5	...do....	+	2+	2+	2+	+	−	−	−
Control serums: [2]									
A	...do....	−	−	−	−	−	−	−	−
No. 1	...do....	−	−	−	−	−	−	−	−
No. 2	...do....	−	−	−	−	−	−	−	−

[1] No antigen in 1:10 dilution.
[2] Control serum A was from laboratory case 6 of our series 13 days after he began work with infected animals, but 67 days before he developed the disease.

TABLE IV.—*Complement fixation test on serums of 5 laboratory cases of tularæmia, using 35 control serums. Antigen used, G 32. Test made June 15, 1921.*

Serum.	Serum dilutions (no antigen in 1 : 10 dilution).								Results.
	1 : 10.	1 : 20.	1 : 40.	1 : 100.	1 : 200.	1 : 400.	1 : 1,000.	1 : 2,000.	
Laboratory cases:									
Case 1	−	4+	3+	+	+	−	−	−	Positive.
Case 2	−	4+	4+	3+	3+	+	−	−	Do.
Case 3	−	4+	4+	4+	4+	4+	−	−	Do.
Case 4	−	4+	4+	4+	4+	4+	+	−	Do.
Case 5	−	4+	4+	4+	3+	2+	−	−	Do.
Control serums:									
A [1]	−	−	−	−	−	−	−	−	Negative.
No. 1 [2]	−	−	−	−	−	−	−	−	Do.
No. 2 [3]	−	−	−	−	−	−	−	−	Do.
No. 3	−	−	−	−	−	−	−	−	Do.
No. 4	−	−	−	−	−	−	−	−	Do.
No. 5	−	−	−	−	−	−	−	−	Do.
No. 6 [4]	4+	+	−	−	−	−	−	−	Negative (Ac).[7]
No. 7 [5]	−	−	−	−	−	−	.....	.....	Negative.
No. 8	−	−	−	−	−	−	.....	.....	Do.
No. 9	−	−	−	−	−	−	.....	.....	Do.
No. 10	−	4+	4+	4+	3+	−	.....	.....	Positive.[8]
No. 11	−	−	−	−	−	−	.....	.....	Negative.
No. 12	−	−	−	−	−	−	.....	.....	Do.
No. 13	−	+	+	2+	+	−	.....	.....	Positive (?).[8]
No. 14	−	−	−	−	−	−	.....	.....	Negative.
No. 15	−	−	−	−	−	−	.....	.....	Do.
No. 16	−	−	−	−	−	−	.....	.....	Do.
No. 17	−	−	−	−	−	−	.....	.....	Do.
No. 18 [6]	+	4+	4+	4+	+	−	.....	.....	Positive.[8]
No. 19	−	−	−	−	−	−	.....	.....	Negative.
No. 20	−	−	−	−	−	−	.....	.....	Do.
No. 21	−	−	−	−	−	−	.....	.....	Do.
No. 22	−	−	−	−	−	−	.....	.....	Do.
No. 23	−	−	−	−	−	−	.....	.....	Do.
No. 24	−	−	−	−	−	−	.....	.....	Do.
No. 25	+	+	+	−	−	−	.....	.....	Negative (Ac).[7]
No. 26	−	−	−	−	−	−	.....	.....	Negative.
No. 27	−	−	−	−	−	−	.....	.....	Do.
No. 28	−	−	−	−	−	−	.....	.....	Do.
No. 29	−	3+	2+	+	−	−	.....	.....	Positive (?).
No. 30	+	−	−	−	−	−	.....	.....	Negative (Ac).[7]
No. 31	+	+	−	−	−	−	.....	.....	Do.
No. 32	−	−	−	−	−	−	.....	.....	Negative.
No. 33	−	−	−	−	−	−	.....	.....	Do.
No. 34	2+	2+	3+	4+	4+	4+	.....	.....	Positive (?) Ac.[7]

[1] Control serum A was taken 48 days after C. W. P. began work with infected animals. He developed tularæmia 32 days after this test.

[2] Control serum 1 is from C. W. C., who had a probable attack of tularæmia more than 10 years ago.

[3] Control serums 2-5 were from other members of laboratory staff who have been slightly exposed to infection.

[4] Control serum 6 was a high titre rabbit antityphoid serum.

[5] The remainder of the serums are from two large local Government hospitals.

[6] Control serum 18 was from a case of typhoid fever (in which *B. typhosus* was isolated), which, at this time, showed a positive Widal.

[7] Ac=anticomplementary.

[8] See Table V.

TABLE V.—*Agglutination tests to determine whether control serums 10, 13, and 18, found positive by the complement fixation test (see Table IV), would be negative by agglutination. Antigen used G 32. Test made June 16, 1921.*

Serum.	Serum dilutions.						Results.
	1:10	1:20	1:40	1:100	1:200	1:400	
Laboratory cases:							
Case 1	4+	4+	3+	+	−	−	Positive.
Case 2	4+	4+	3+	2+	−	−	Do.
Case 3	+	2+	4+	4+	2+	−	Do.
Case 4	4+	4+	4+	4+	3+	−	Do.
Case 5	+	3+	4+	3+	2+	−	Do.
Control serums:							
A	−	−	−	−	−	−	Negative.
No. 1	−	−	+	2+	−	−	Slightly positive (?).
No. 2	−	−	−	−	−	−	Negative.
No. 3	−	−	−	−	−	−	Do.
No. 4	−	−	−	−	−	−	Do.
No. 5	−	−	−	−	−	−	Do.
No. 10	−	−	−	−	−	−	Do.
No. 13	−	−	−	−	−	−	Do.
No. 18	−	−	−	−	−	−	Do.

TABLE VI.—*Agglutination test made Aug. 5, 1921, on serum of laboratory case 6 taken on the nineteenth day of his illness. This patient had furnished negative control serum A 51 and 87 days previously. (See Tables III, IV, and V.) All serums taken Aug. 5 and heated 30 minutes at 55° C. before using. Antigen used G 32.*

Serums.	Serum dilutions.							Results.
	No serum.	1:20	1:40	1:80	1:200	1:400	1:800	
Laboratory cases:								
Case 6	−	2+	3+	2+	2+	+	−	Positive.
Case 3	−	2+	2+	2+	+	−	−	Do.
Case 4	−	3+	3+	2+	+	−	−	Do.
Control serums:								
No. 1	−	−	−	−	−	−	−	Negative.
No. 2	−	−	−	−	−	−	−	Do.
No. 3	−	−	−	−	−	−	−	Do.
No. 4	−	−	−	−	−	−	−	Do.
No. 5	−	−	−	−	−	−	−	Do.
No. 6	−	−	−	−	−	−	−	Do.

RECORDS OF THE SMALL SICK-BENEFIT ASSOCIATION AS A SOURCE OF STATISTICS FOR THE FACTORY MEDICAL DEPARTMENT.[1]

By DEAN K. BRUNDAGE, United States Public Health Service.

The keeping of adequate sickness records for the employees of an industrial establishment is no easy proposition. As a general rule the industrial physician finds it exceedingly difficult to obtain the fundamental information required for efficient administration of the factory health department. How, for example, can the ailments causing disability be ascertained for employees absent from work on account of illness? How can trustworthy sickness *rates* be obtained when such rates require as the dividend in the expression, *all* cases

[1] From the Statistical Office, United States Public Health Service.